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(54) **Tumor necrosis related receptor, TR5**

(57) TR5 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR5 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia,

autoimmune diseases (eg inflammatory bowel disease, graft vs. host disease), transplantation, infection, stroke, ischemia, acute respiratory disease syndrome, psoriasis, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atherosclerosis, and Alzheimers disease among others, and diagnostic assays for such conditions.

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Description**CROSS REFERENCE TO RELATED APPLICATION**

5 This is a continuation in part application of U.S. serial no. 08/795,910 filed February 5, 1997, which is herein incorporated by reference in its entirety.

FIELD OF INVENTION

10 This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Tumor necrosis factor receptor (TNF-R) related protein, hereinafter referred to as TR5. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response

20 For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

25 Among the ligands there are included TNF-a, lymphotoxin-a (LT-a, also known as TNF-b), LT-b (found in complex heterotrimer LT-a2-b), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF)). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., *Biologicals*, 22:291-295 (1994)).

30 Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., *supra*).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., *Nature* 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause 35 an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., *Science* 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innervation of peripheral structures (Lee, K.F. et al, *Cell* 69:737 (1992)).

40 TNF and LT-a are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-a, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-a are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., *Science* 264:667-668 (1994)). Mutations in the p55 Receptor cause 45 increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., *Cell* 74:845 (1993)).

50 The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

This indicates that these Tumor necrosis factor receptors (TNF-R) have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of Tumor necrosis factor receptor (TNF-R) family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, 55 including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders),

atherosclerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TR5 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TR5 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atherosclerosis, and Alzheimers disease among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TR5 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TR5 activity or levels.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide and deduced amino acid sequence of human TR5. SEQ ID NOS: 1 and 2.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TR5" refers generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"TR5 activity or TR5 polypeptide activity" or "biological activity of the TR5 or TR5 polypeptide" refers to the metabolic or physiologic function of said TR5 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TR5.

"TR5 polypeptides" refers to polypeptides with amino acid sequences sufficiently similar to TR5 sequences, preferably exhibiting at least one biological activity of the TR5.

"TR5 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"TR5 polynucleotides" refers to polynucleotides containing a nucleotide sequence which encodes a TR5 polypeptide or fragment thereof, or a nucleotide sequence which has at least 80% identity to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or the corresponding fragment thereof, or a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polyeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may

contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS-STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., "Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", Meth Enzymol (1990) 182:626-646 and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging", Ann NY Acad Sci (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., SIAM J Applied Math (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J Applied Math (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., et al., Nucleic Acids Research (1984) 12(1):387), BLASTP, BLASTN, FASTA (Altschul, S.F. et al., J.Molec Biol (1990) 215:403).

Polypeptides of the Invention

The TR5 polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as TR5 polypeptides and which have at least 80% identity to the polypeptide of SEQ ID NO:2 or the relevant portion and more preferably at least 85% identity, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO:2.

The TR5 polypeptides may be in the form of the "mature" protein or may be a part of larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional

sequence for stability during recombinant production.

Biologically active fragments of the TR5 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TR5 polypeptides. As with TR5 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TR5 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TR5 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Biologically active fragments are those that mediate TR5 activity, including those with a similar activity, or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Thus, the polypeptides of the invention include polypeptides having an amino acid sequence at least 80% identical to that of SEQ ID NO:2 or fragments thereof with at least 80% identity to the corresponding fragment of SEQ ID NO:2. Preferably, all of these polypeptides retain the biological activity of the TR5, including antigenic activity. Included in this group are variants of the defined sequence and fragments. Preferred variants are those that vary from the referents by conservative amino acid substitutions— i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TR5 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to isolated polynucleotides which encode the TR5 polypeptides and polynucleotides closely related thereto.

TR5 of the invention is structurally related to other proteins of the Tumor necrosis factor receptor (TNF-R), as shown by the results of sequencing the cDNA encoding human TR5. The cDNA sequence contains an open reading frame encoding a protein of 299 amino acids with a deduced molecular weight of 31.8 kDa. TR5 of Figure 1 (SEQ ID NO:2) has about 25.6% identity (using BESTFIT (from GCG suite of Programs)) in 274 amino acid residues with Human low affinity Nerve Growth Factor receptor (LNGF-R) (SWISSPROT Accession: NGFR_HUMAN; D. Johnson et al., Cell 47:545-554 (1986)). Furthermore, TR5 (SEQ ID NO:2) is 22.4% identical to low affinity rat Nerve Growth Factor Receptor over 281 amino acid residues (SWISSPROT Accession: NGFR_RAT; M.J. Radeke et al., Nature 325:593-597 (1987)). TR5 gene of Figure 1 (SEQ ID NO:1) has about 89% identity (using BLAST) in 37 nucleotide residues with H. Sapiens CpG island DNA genomic MseI fragment (Genbank Accession Z54987; S.H Cross et al., Nature Genet. 6: 236-244(1994)). Furthermore, TR5 is 52% identical to O. cuniculus KAP4C mRNA over 195 nucleotide base residues (Genbank Accession X80035; B.C. Powell et al., Differentiation 58:227-232 (1995)).

One polynucleotide of the present invention encoding TR5 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of prostate, endothelial cells, IL-1beta treated smooth muscle cells, fetal liver spleen cells, and pregnant uterus using the expressed sequence tag (EST) analysis (Adams, M.D., et al., Science (1991) 252:1651-1656; Adams, M.D. et al., Nature, (1992)355:632-634; Adams, M.D., et al., Nature (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

Thus, the nucleotide sequence encoding TR5 polypeptides may be identical over its entire length to the coding sequence in Figure 1 (SEQ ID NO:1), or may be a degenerate form of this nucleotide sequence encoding the polypeptide of SEQ ID NO:2, or may be highly identical to a nucleotide sequence that encodes the polypeptide of SEQ ID NO:2. Preferably, the polynucleotides of the invention contain a nucleotide sequence that is highly identical, at least 80% identical, with a nucleotide sequence encoding a TR5 polypeptide, or at least 80% identical with the encoding nucleotide

sequence set forth in Figure 1 (SEQ ID NO:1) or at least 80% identical to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TR5 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., Proc Natl Acad Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Among particularly preferred embodiments of the invention are polynucleotides encoding TR5 polypeptides having the amino acid sequence of set out in Figure 1 (SEQ ID NO:2) and variants thereof.

Further preferred embodiments are polynucleotides encoding TR5 variants that have the amino acid sequence of the TR5 polypeptide of Figure 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

Further preferred embodiments of the invention are polynucleotides that are at least 80% identical over their entire length to a polynucleotide encoding the TR5 polypeptide having the amino acid sequence set out in Figure 1 (SEQ ID NO:2), and polynucleotides which are complementary to such polynucleotides. In this regard, polynucleotides at least 80% identical over their entire length to the same are particularly preferred, and those with at least 90% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides of the invention, which are sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TR5 polypeptide and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the TR5 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 70% identical, preferably 80% identical, more preferably 90% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., BASIC METHODS IN MOLECULAR BIOLOGY (1986) and Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, E. coli, Streptomyces and Bacillus subtilis cells; fungal cells, such as yeast cells and Aspergillus cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic

elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL (supra).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TR5 polypeptide is to be expressed for use in screening assays, the polypeptide may be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TR5 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

TR5 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TR5 polynucleotides for use as diagnostic reagents. Detection of mutated form of TR5 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TR5. Individuals carrying mutations in the TR5 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TR5 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers et al., Science (1985) 230: 1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton et al., Proc Natl Acad Sci USA (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotide probes comprising TR4 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atherosclerosis, and Alzheimers disease through detection of mutation in the TR5 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atherosclerosis, and Alzheimers disease can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of TR5 polypeptide or TR5 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TR5 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The gene for TR5 has been localized to chromosome 8 between bands 8p12 and 8p22, approximately 42-60cM from the top (p terminus) of chromosome 8.

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TR5 polypeptides. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TR5 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole et al., MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TR5 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atherosclerosis, and Alzheimers disease among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TR5 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (eg inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atherosclerosis, and Alzheimers disease among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TR5 gene via a vector directing expression of TR5 polypeptide in vivo in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TR5 polypeptide wherein the composition comprises a TR5 polypeptide or TR5 gene. The vaccine formulation may further comprise a suitable carrier. Since TR5 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition.

requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

We have now discovered that TL2 of SEQ ID NO: 3 (otherwise known as TRAIL, TWiley SR, et al., Immunity (6): 673-682 (1995)) is a ligand of TR5. Thus, the TR5 polypeptide of the present invention, and one of its ligands, TL2 may be employed in a screening process for compounds which bind the receptor, or its ligand, and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention, or its ligand TL2. Thus, polypeptides of the invention may be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).

TR5 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate TR5 on the one hand and which can inhibit the function of TR5 or remove TR5 expressing cells on the other hand. Antagonists, or agents which remove TR5 expressing cells, may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g. lymphoproliferative disorders), atherosclerosis, and Alzheimers disease. Agonists can be employed for therapeutic and prophylactic purposes for such conditions responsive to activation of T cells and other components of the immune system, such as for treatment of cancer and AIDS. However, agonists can also be employed for inappropriate stimulation of T cells and other components of the immune system which leads to down modulation of immune activity with therapeutic or prophylactic application for conditions such, as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g. inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury,, Bone diseases,, atherosclerosis, and Alzheimers disease.

Candidate compounds may be identified using assays to detect compounds which inhibit binding of TL2 to TR5 in either cell-free or cell based assays. Suitable cell-free assays may be readily determined by one of skill in the art. For example, an ELISA format may be used in which purified TR5, or a purified derivative of TR5, containing the extracellular domain of TR5, is immobilized on a suitable surface, either directly or indirectly (e.g., via an antibody to TR5) and candidate compounds are identified by their ability to block binding of purified TL2 to TR5. The binding of TL2 to TR5 could be detected by using a label directly or indirectly associated with TL2. Suitable detection systems include the streptavidin horseradish peroxidase conjugate, or direct conjugation by a tag, e.g., fluorescein. Conversely, purified TL2 may be immobilized on a suitable surface, and candidate compounds identified by their ability to block binding of purified TR5 to TL2. The binding of TR5 to TL2 could be detected by using a label directly or indirectly associated with TR5. Many other assay formats are possible that use the TR5 protein and its ligands.

Suitable cell based assays may be readily determined by one of skill in the art. In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, Drosophila or E. coli. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a known ligand, such as TL2, or test compound to observe binding, or stimulation or inhibition of a functional response. The assays may simply test binding of candidate compound wherein adherence to the cells bearing the receptor is detected by means of label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor, such as the ligand TL2. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor or its ligand (e.g. TL2) using detection systems appropriate to the cells bearing the receptor or its ligand and fusion proteins thereof at their surfaces. Typical fusion partners include fusing the extracellular domain of the receptor or ligand with the intracellular tyrosine kinase domain of a second receptor. Inhibitors of activation are generally assayed in the presence of a known agonist, such as the ligand TL2, and the effect on activation by the agonist by the presence of the candidate compound is observed. Standard methods for conducting such screening assays are well understood in the art.

Examples of potential TR5 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the TR5, e.g., a fragment of the ligand TL2, or small molecules which bind to the receptor, or its ligand, but do not elicit a response, so that the activity of the receptor is prevented. Examples of potential TR5 agonists include antibodies that bind to TR5, its ligand, such as TL2, or derivatives thereof, and small molecules that bind to TR5. These agonists will elicit a response mimicking all or part of the response induced by contacting the

native ligand.

Alternatively, TR5 may be expressed as a soluble protein, including versions which fuse all or part of TR5 with a convenient partner peptide for which detection reagents are available, eg TR5-IgG fusions, and used in a solid state or solution phase binding assay. For example, the soluble TR5 can be used to detect agonist or antagonist binding directly through changes that can be detected experimentally, eg surface plasmon resonance, nuclear magnetic resonance spectrometry, sedimentation, calorimetry. The soluble TR5 can be used to detect agonist or antagonist binding indirectly by looking for competition of the candidate agonist or antagonist with a ligand whose binding can be detected. Ligand detection methods include antibody recognition, modification of the ligand via radioactive labeling, chemical modification (eg biotinylation), fusion to an epitope tag. Methods include ELISA based assays, immunoprecipitation and scintillation proximity.

Assays similar to those described above using soluble or membrane bound TR5 may also be used to identify and purify the natural ligand(s) of TR5. These ligands may be agonists or antagonists of the receptor.

Examples of potential TR5 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, etc., as the case may be, of the TR5 polypeptide, e.g., a fragment of the ligands, substrates, receptors, or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

The TR5 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of TR5 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of TR5 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents (i.e. antagonists or agonists) which may inhibit or enhance the production of TR5 from suitably manipulated cells or tissues.

Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of TR5 polypeptide activity.

If the activity of TR5 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the TR5 polypeptide, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of TR5 polypeptides still capable of binding the ligand in competition with endogenous TR5 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the TR5 polypeptide.

In still another approach, expression of the gene encoding endogenous TR5 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, J Neurochem (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee et al., Nucleic Acids Res (1979) 6:3073; Cooney et al., Science (1988) 241:456; Dervan et al., Science (1991) 251:1360. These oligomers can be administered per se or the relevant oligomers can be expressed in vivo.

For treating abnormal conditions related to an under-expression of TR5 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of compound which activates TR5 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TR5 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells in vivo and expression of the polypeptide in vivo. For overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

Peptides, such as the soluble form of TR5 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers

include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide such as a DNA or RNA, to encode a polypeptide ex vivo, and for example, by the use of retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

An EST (EST# 213397; Project ID HPRCB54) derived from a cDNA library made from human prostate and deposited in a commercial EST database was found to have sequence similarity to the human TNF receptor. A search through several overlapping ESTs indicated that this represented the 5' most EST of the assembly and so it was completely sequenced. Analysis of the 1410 nucleotide cDNA sequence indicated that it encoded a complete open reading frame for a novel member of the TNF receptor superfamily and was named TR5. The predicted protein is 300 amino acids long, and contains an amino-terminal hydrophobic signal sequence required for secretion. Cleavage is predicted to occur at the amino acid #65/66 junction. This means that the signal sequence is amino acids 1-65 (or 41-65 if translation starts at the second Methionine at position 41), and the mature protein is amino acids 66-299 (ie 234 amino acids). Which of the initiation sites is used is not known. Furthermore, the protein contains a carboxy terminal hydrophobic region and lacks a cytoplasmic tail, which is characteristic of proteins which are cleaved and attached to the extracellular membrane surface through a glycosylphosphatidylinositol (GPI) linkage. In addition to being found on the surface of cells, GPI-linked proteins can also be enzymatically released from cells as secreted proteins through cleavage of the GPI linkage. Comparison of TR5 with other TNF receptor family proteins indicates that it has at least two complete and one partial copy of the cysteine-rich repeats characteristic of the extracellular domains of this family. Between these repeat regions characteristic of the ligand binding domain of the TNF receptor family and the carboxy-terminus is a series of 5 repeats of an approximately 15 amino acid sequence rich in Glutamate (E), Threonine (T), Serine (S), Proline (P), Alanine (A) and Glycine (G). Such sequences are commonly associated with regions rich in O-glycosylation on Serine and Threonine. TR5 is most similar to the extracellular domain of the low affinity NGF receptor, which also has a membrane proximal O-glycosylation region.

Chromosomal location.

The complete TR5 cDNA obtained above was searched against Genbank and identified an exact match between the 3' untranslated region (3' UTR) and a Sequence Tagged Site (STS) (Genbank Accession #G23178; human STS WI-11701). STS sequences are derived from PCR primer pairs often selected from the 3' UTRs of randomly sequenced cDNAs and subsequently used to map their chromosomal location via radiation hybrid mapping and other physical mapping methods. This STS had previously been chromosomally localized to a specific region of chromosome 8 be-

tween 8p12 and 8p22 (42-60cM from top of chromosome 8 between markers D8S298 and D8S505). No known TNF receptor family members are known to map to this chromosomal region.

Northern blot of TR5.

Various tissues and cell lines were screened for mRNA expression by Northern blot. RNA was prepared from cells and cell lines using Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH), run in denaturing agarose gels (Sambrook et al., Molecular Cloning: a laboratory manual, 2nd Ed. Cold Spring Harbor Lab Press, NY (1989)) and transferred to Zeta-probe nylon membrane (Biorad, Hercules, CA.) via vacuum blotting in 25mM NaOH for 90 min. After neutralization for 5-10 minutes with 1M tris-HCl, pH 7.5 containing 3M NaCl, the blots were prehybridized with 50% formamide, 8% dextran sulfate, 6XSSPE, 0.1%SDS and 100mg/ml of sheared and denatured salmon sperm DNA for at least 30 min. At 42°C. cDNA probes were labeled with 32P-CTP by random priming (Statagene, La Jolla, CA), briefly denatured with 0.25M NaOH and added to the prehybridization solution. After a further incubation for at least 24h at 42°C, the blots were washed in high stringency conditions and exposed to X-ray film.

TR5 was expressed in resting CD19+ B lymphocytes and CD8+ and CD4+ T lymphocytes. However, RNA was not detectable 48 hours following activation by PHA+PMA of CD8+ and CD4+ T lymphocytes. Highest expression of TR5 message was seen in bone marrow. High expression was also detected in monocytes and aortic endothelial cells.

RNA was in KG1a hematopoietic cell line, in HL60 following DMSO treatment (which leads to granulocytic differentiation), and in U937 and THP1 cells following PMA treatment (which leads to monocytic differentiation). RNA was also detectable in HeLa cells.

Multiple forms of RNA (4) were detected ranging in sizes from 1.6 to 5 kb. The major forms expressed in lymphocytes are 1.6 and 2.5 kb in sizes. The major forms expressed in endothelial cells are the 1.6, 3.5 and 5 kb RNAs.

Annex to the description

SEQUENCE LISTING

5

(1) GENERAL INFORMATION

10

(i) APPLICANT: SmithKline Beecham Corporation

15

(ii) TITLE OF THE INVENTION: Tumor Necrosis Related Receptor, TR5

(iii) NUMBER OF SEQUENCES: 3

20

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: F J Cleveland & Company

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25

(C) CITY: London

(D) COUNTRY:

(E) COUNTRY: United Kingdom

30

(F) POST CODE: WC2A 1JQ

(v) COMPUTER READABLE FORM:

35

(A) MEDIUM TYPE: Diskette

(B) COMPUTER IBM Compatible

(C) OPERATING SYSTEM: DOS

40

(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

45

(A) APPLICATION NUMBER Unknown

(B) FILING DATE: 28 July 1997

(C) CLASSIFICATION:

50

(vii) PRIOR APPLICATION DATA:

55

(A) APPLICATION NUMBER 08/795,910

(B) FILING DATE: February 5, 1997

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: CRUMP, Julian Richard John
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(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: +44 171 405 5875
 (B) TELEFAX: +44 171 831 0749
 (C) TELEX:

(x) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1410 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CACGAGCTC TCCACGCGCA CGAACTCAGC CAACGATTTT TGATAGATTT TTGGGAGTTT	60
GACCAGAGAT GCAAGGGGTG AAGGAGCGCT TCCTACCGTT AGGGAAGTCT GGGGACAGAG	120
CGCCCCGGCC GCCTGATGGC CGAGGCAGGG TCGGACCCAG GACCCAGGAC GGCGT CGGGA	180
ACCATACCAT GGCCCGGATC CCCAAGACCC TAAAGTTCGT CGT CGT CAT C GT CGCGGTCC	240
TGCTGCCAGT CCTAGCTTAC TCTGCCACCA CTGCCCGGCA GGAGGAAGTT CCCCAGCAGA	300
CAGTGGCCCC ACAGCAACAG AGGCACAGCT TCAAGGGGGA GGAGTGTCCA GCAGGATCTC	360
ATAGATCAGA ACATACTGGA GCCTGTAACC CGTGCACAGA GGGTGTGGAT TACACCAACG	420
CTTCCAACAA TGAACCTTCT TGCTTCCCAT GTACAGTTTG TAAATCAGAT CAAAAACATA	480
AAAGTTCTG CACCATGACC AGAGACACAG TGTGTAGTG TAAAGAAGGC ACCTTCCGGA	540
ATGAAAACCTC CCCAGAGATG TGCCGGAAGT GTAGCAGGTG CCCTAGTGGG GAAGTCCAAG	600

TCAGTAATTG TAGGTCTGG GATGATATCC AGTGTGTTGA AGAATTGGT GCCAATGCCA 660
 CTGTGGAAAC CCCAGCTGCT GAAGAGACAA TGAACACCAG CCCGGGSACT CCTGCCCCAG 720
 5 CTGCTGAAGA GACAATGAAC ACCAGCCCAG GGAATCCTGC CCCAGCTGCT GAAGAGACAA 730
 TGACCACCAG CCCGGGSACT CCTGCCCCAG CTGCTGAAGA GACAATGACC ACCAGCCCAG 840
 GGACTCCTGC CCCAGCTGCT GAAGAGACAA TGACCACCAG CCCGGGSACT CCTGCCTCTT 900
 10 CTGATTACCT CTGATCCACC ATCGTAGGGA TCATAGTTCT AATTGTGCTT CTGATTGTGT 960
 TTGTTTGAAA GACTTCACCTG TGGAAGAAAT TCCTTCCTTA CCTGAAAGGT TCAGGTAGGC 1020
 GCTGGCTGAG GGGGGGGGGC CCTGGACACT CTCTGCCCTG CTTCCCTCTG CTGTGTTCCC 1080
 ACAGACAGAA AGCCTGCCC CTGCCCCAAG TCCTGCTGT C TCAGCCCTGG CTCTATCTTC 1140
 15 CTCTTGTGA TCGTCCCATC CCCACATCCC GTGCACCCCC CAGGACCCTG GTCTCATCAG 1200
 TCCCTCTCCT GGAGCTGGGG GTCCACACAT CTCCAGCCA ACTCCAAGAG GGCAGGGCCA 1260
 GTTCTCCCA TCTTCAGGCC CAGCCAGGCA GGGGCGAGTC GGCTCCTCAA CTGGGTGACA 1320
 20 AGGGTGAGGA TGAGAAGTGG TCAGCGGAT TATTGAGCCT TGGTCAGAGC AGAACACAGA 1380
 GATTTTCCGT GAAAAAATAA AAAAAAATAA 1410

25

(2) INFORMATION FOR SEQ ID NO: 2:

30

(i) SEQUENCE CHARACTERISTICS:

35

(A) LENGTH: 299 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

45

Met Gln Gly Val Lys Glu Arg Phe Leu Pro Leu Gly Asn Ser Gly Asp
 1 5 10 15
 Arg Ala Pro Arg Pro Pro Asp Gly Arg Gly Arg Val Arg Pro Arg Thr
 20 25 30
 Gln Asp Gly Val Gly Asn His Thr Met Ala Arg Ile Pro Lys Thr Leu
 35 40 45

55

EP 0 867 509 A2

5 Lys Phe Val Val Val Ile Val Ala Val Leu Leu Pro Val Leu Ala Tyr
 50 55 60
 Ser Ala Thr Thr Ala Arg Gln Glu Glu Val Pro Gln Gln Thr Val Ala
 65 70 75 80
 Pro Gln Gln Gln Arg His Ser Phe Lys Gly Glu Glu Cys Pro Ala Gly
 10 85 90 95
 Ser His Arg Ser Glu His Thr Gly Ala Cys Asn Pro Cys Thr Glu Gly
 100 105 110
 Val Asp Tyr Thr Asn Ala Ser Asn Asn Glu Pro Ser Cys Phe Pro Cys
 15 115 120 125
 Thr Val Cys Lys Ser Asp Gln Lys His Lys Ser Ser Cys Thr Met Thr
 130 135 140
 Arg Asp Thr Val Cys Gln Cys Lys Glu Gly Thr Phe Arg Asn Glu Asn
 20 145 150 155 160
 Ser Pro Glu Met Cys Arg Lys Cys Ser Arg Cys Pro Ser Gly Glu Val
 25 165 170 175
 Gln Val Ser Asn Cys Thr Ser Trp Asp Asp Ile Gln Cys Val Glu Glu
 180 185 190
 Phe Gly Ala Asn Ala Thr Val Glu Thr Pro Ala Ala Glu Glu Thr Met
 30 195 200 205
 Asn Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Asn
 210 215 220
 Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr
 35 225 230 235 240
 Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser
 40 245 250 255
 Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser Pro
 260 265 270
 Gly Thr Pro Ala Ser Ser His Tyr Leu Ser Cys Thr Ile Val Gly Ile
 45 275 280 285
 Ile Val Leu Ile Val Leu Leu Ile Val Phe Val
 290 295

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 279 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

15 Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys Val Leu
 1 5 10 15
 Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala Val Thr
 20 25 30
 20 Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys Tyr Ser
 35 40 45
 Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr Trp Asp
 50 55 60
 25 Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val Lys Trp
 65 70 75 80
 Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser Glu Glu
 85 90 95
 30 Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val
 100 105 110
 Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg
 115 120 125
 35 Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala
 130 135 140
 Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser
 145 150 155 160
 40 Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu
 165 170 175
 Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu
 180 185 190
 45 Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile
 195 200 205
 Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala
 210 215 220
 50 Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile
 225 230 235 240
 Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val
 245 250 255
 55 Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe

260
 Phe Gly Ala Phe Leu Val Gly
 275

Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or the corresponding fragment thereof; or a nucleotide sequence complementary to said nucleotide sequence.
2. The polynucleotide of claim 1 which is DNA or RNA.
3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.
4. The polynucleotide of claim 3 wherein said nucleotide sequence is contained in SEQ ID NO:1.
5. The polynucleotide of claim 1 wherein said encoding nucleotide sequence encodes the polypeptide of SEQ ID NO:2 or a fragment thereof.
6. A polynucleotide probe or primer comprising at least 15 contiguous nucleotides of the polynucleotide of claim 3.
7. A DNA or RNA molecule comprising an expression system wherein said expression system is capable of producing a TR5 polypeptide or a fragment thereof having at least 80% identity with a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or said fragment when said expression system is present in a compatible host cell.
8. A host cell comprising the expression system of claim 7.
9. A process for producing a TR5 polypeptide or fragment comprising culturing a host of claim 8 and under conditions sufficient for the production of said polypeptide or fragment.
10. The process of claim 9 wherein said polypeptide or fragment is expressed at the surface of said cell.
11. Cells produced by the process of claim 10.
12. The process of claim 9 which further includes recovering the polypeptide or fragment from the culture.
13. A process for producing a cell which produces a TR5 polypeptide or a fragment thereof comprising transforming or transfecting a host cell with the expression system of claim 7 such that the host cell, under appropriate culture conditions, produces a TR5 polypeptide or fragment.
14. A TR5 polypeptide or a fragment thereof comprising an amino acid sequence which is at least 80% identical to the amino acid sequence contained in SEQ ID NO:2.
15. The polypeptide of claim 14 which comprises the amino acid sequence of SEQ ID NO:2, or a fragment thereof.
16. A TR5 polypeptide or fragment prepared by the method of claim 12.
17. An antibody immunospecific for the TR5 polypeptide of claim 14.
18. A method for the treatment of a subject in need of enhanced TR5 polypeptide activity comprising:
 - (a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or
 - (b) providing to the subject TR5 polynucleotide in a form so as to effect production of said polypeptide activity

in vivo.

19. A method for the treatment of subject having need to inhibit TR5 polypeptide activity comprising:

- 5 (a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or
(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or
(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate, or receptor.

10 20. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TR5 polypeptide in a subject comprising:

- 15 (a) determining the presence or absence of mutation in the nucleotide sequence encoding said TR5 polypeptide in the genome of said subject; and/or
(b) analyzing for the presence or amount of the TR5 polypeptide expression in a sample derived from said subject.

20 21. A method for identifying compounds which bind to TR5 polypeptide comprising:

- (a) contacting cells of claim 11 with a candidate compound; and
(b) assessing the ability of said candidate compound to bind to said cells.

25 22. The method of claim 21 which further includes determining whether the candidate compound effects a signal generated by activation of the TR5 polypeptide at the surface of the cell, wherein a candidate compound which effects production of said signal is identified as an agonist.

23. An agonist identified by the method of claim 22.

30 24. The method of claim 21 which further includes contacting said cell with a known agonist for said TR5 polypeptide; and determining whether the signal generated by said agonist is diminished in the presence of said candidate compound, wherein a candidate compound which effects a diminution in said signal is identified as an antagonist for said TR5 polypeptide.

35 25. An antagonist identified by the method of claim 24.

FIGURE 1

10 30 50

CACGAGCCTCTCCACGCGCACGAACCTCAGCCAACGATTTCTGATAGATTTTTGGGAGTTT

70 90 110

GACCAGAGATGCAAGGGGTGAAGGAGCGCTTCTACCGTTAGGGAACTCTGGGGACAGAG
MetGlnGlyValLysGluArgPheLeuProLeuGlyAsnSerGlyAspArgA

130 150 170

CGCCCCGGCCGCTGATGGCCGAGGCGGTGCGACCCAGGACCCAGGACGGCGTCGGGA
laProArgProProAspGlyArgGlyArgValArgProArgThrGlnAspGlyValGlyA

190 210 230

ACCATACCATGGCCCGSATCCCCAAGACCCTAAAGTTCGTCGTCGTCATCGTCGCGGTCC
snHisThrMetAlaArgIleProLysThrLeuLysPheValValValIleValAlaValL

250 270 290

TGCTGCCAGTCTTAGCTTACTCTGCCACCACTGCCCGGCAGGAGGAAGTTCCCCAGCAGA
euLeuProValLeuAlaTyrSerAlaThrThrAlaArgGlnGluGluValProGlnGlnT

310 330 350

CAGTGGCCCCACAGCAACAGAGGCACAGCTTCAAGGGGGAGGAGTGTCAGCAGGATCTC
hrValAlaProGlnGlnGlnArgHisSerPheLysGlyGluGluCysProAlaGlySerH

370 390 410

ATAGATCAGAACATACTGGAGCCTGTAACCCGTGCACAGAGGTGTGGATTACACCAACG
isArgSerGluHisThrGlyAlaCysAsnProCysThrGluGlyValAspTyrThrAsnA

430 450 470

CTTCCAACAATGAACCTTCTTGCTTCCCATGTACAGTTTGTAATCAGATCAAAAACATA
laSerAsnAsnGluProSerCysPheProCysThrValCysLysSerAspGlnLysHisL

490 510 530
 AAAGTTCCTGCACCATGACCAGAGACACAGTGTGTCAGTGTAAGAAGGCACCTTCCGGA
 ysSerSerCysThrMetThrArgAspThrValCysGlnCysLysGluGlyThrPheArgA

550 570 590
 ATGAAAACTCCCCAGAGATGTCCCGGAAGTGTAGCAGGTGCCCTAGTGGGGAAGTCCAAG
 snGluAsnSerProGluMetCysArgLysCysSerArgCysProSerGlyGluValGlnV

610 630 650
 TCAGTAATTGTACGTCCTGGGATGATATCCAGTGTGTTGAAGAATTGGTGCCAATGCCA
 alSerAsnCysThrSerTrpAspAspIleGlnCysValGluGluPheGlyAlaAsnAlaT

670 690 710
 CTGTGGAAACCCAGCTGCTGAAGAGACAATGAACACCAGCCCGGGGACTCCTGCCCCAG
 hrValGluThrProAlaAlaGluGluThrMetAsnThrSerProGlyThrProAlaProA

730 750 770
 CTGCTGAAGAGACAATGAACACCAGCCAGGGACTCCTGCCCCAGCTGCTGAAGAGACAA
 laAlaGluGluThrMetAsnThrSerProGlyThrProAlaProAlaAlaGluGluThrM

790 810 830
 TGACCACCAGCCCGGGGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCCGG
 etThrThrSerProGlyThrProAlaProAlaAlaGluGluThrMetThrThrSerProG

850 870 890
 GGACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTCCTGCCTCTT
 lyThrProAlaProAlaAlaGluGluThrMetThrThrSerProGlyThrProAlaSers

910 930 950
 CTCATTACCTCTCATGCACCATCGTAGGGATCATAGTTCTAATTGTGCTTCTGATTGTGT
 erHisTyrLeuSerCysThrIleValGlyIleIleValLeuIleValLeuLeuIleValP

970 990 1010
TTGTTTGAAAGACTTCACTGTGGAAGAAATTCCTTCCTTACCTGAAAGGTTTCAGGTAGGC
heValEnd

1030 1050 1070
GCTGGCTGAGGGCGGGGGGCGCTGGACACTCTCTGCCCTGCCTCCCTCTGCTGTGTTCCC

1090 1110 1130
ACAGACAGAAACGCCTGCCCCCTGCCCCAAGTCCTGGTGTCTCCAGCCTGGCTCTATCTTC

1150 1170 1190
CTCCTTGTGATCGTCCCATCCCCACATCCCGTGCACCCCCCAGGACCCTGGTCTCATCAG

1210 1230 1250
TCCCTCTCCTGGAGCTGGGGGTCCACACATCTCCCAGCCAAGTCCAAGAGGGCAGGGCCA

1270 1290 1310
GTTCCCTCCCATCTTCAGGCCCAGCCAGGCAGGGGGCAGTCGGCTCCTCAACTGGGTGACA

1330 1350 1370
AGGGTGAGGATGAGAAGTGGTCACGGGATTTATTTCAGCCTTGGTCAGAGCAGAACACAGA

1390 1410
GATTTTCCGTGAAAAAAAAAAAAAAAAAAAA

(19)



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(54) **Tumor necrosis related receptor, TR5**

(57) TR5 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TR5 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia,

autoimmune diseases (eg inflammatory bowel disease, graft vs. host disease), transplantation, infection, stroke, ischemia, acute respiratory disease syndrome, psoriasis, restenosis, brain injury, AIDS, Bone diseases, cancer (eg lymphoproliferative disorders), atherosclerosis, and Alzheimers disease among others. and diagnostic assays for such conditions.

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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 98 30 0827 shall be considered, for the purposes of subsequent proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	HILLIER L ET AL: "Homo sapiens cDNA clone 504745 5' (accession number AA150849)" EMBL SEQUENCE DATABASE, 15 December 1996, XP002076202 Heidelberg, Germany * the whole document *	1-8	C12N15/12 C07K14/715 C07K16/28 C12N5/10 C12Q1/68 G01N33/68 A61K38/17
X	HILLIER L ET AL: "Homo sapiens cDNA clone 504745 3' (accession number AA150541)" EMBL SEQUENCE DATABASE, 15 December 1996, XP002076203 Heidelberg, Germany * the whole document *	1-8	
P,X	PAN G ET AL: "AN ANTAGONIST DECOY RECEPTOR AND A DEATH DOMAIN-CONTAINING RECEPTOR FOR TRAIL" SCIENCE, vol. 277, 8 August 1997, pages 815-818, XP002065147 * the whole document *	1-17, 20-22	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C07K C12N C12Q G01N A61K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely .</p> <p>Claims searched incompletely .</p> <p>Claims not searched .</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		2 September 1998	Oderwald, H
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone</p> <p>Y : particularly relevant if combined with another document of the same category</p> <p>A : technological background</p> <p>O : non-written disclosure</p> <p>P : intermediate document</p> <p>T : theory or principle underlying the invention</p> <p>E : earlier patent document, but published on, or after the filing date</p> <p>D : document cited in the application</p> <p>L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p>			

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INCOMPLETE SEARCH
SHEET C

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Although claims 18 and 19 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Claim(s) not searched:
23,25

Reason for the limitation of the search:

Claims 23 and 25 have not been searched due to the lack of sufficient characterisation of the claimed compounds.



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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
P,X	SHERIDAN J P ET AL: "CONTROL OF TRAIL-INDUCED APOPTOSIS BY A FAMILY OF SIGNALING AND DECOY RECEPTORS" SCIENCE, vol. 277, 8 August 1997, pages 818-821, XP002065023 * the whole document *	1-17, 20-22	
A	PITTI R M ET AL: "INDUCTION OF APOPTOSIS BY APO-2 LIGAND, A NEW MEMBER OF THE TUMOR NECROSIS FACTOR CYTOKINE FAMILY" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 22, 31 May 1996, pages 12687-12690, XP002065021 * the whole document *		TECHNICAL FIELDS SEARCHED (Int.Cl.6)

EPO FORM 1500 02/92 (P4/C10)